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# Studies in soil bacteriology. I[1]).

## Nitrification in soils and in solutions.

[Contributed from the North Carolina Agricultural Experiment Station West Raleigh, N. C., U. S. A.]

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### Introductory.

Following the lead of Robert Koch in 1881 in the utilization of his gelatine plate method for enumeration of the bacterial flora of various soils, came a vast number of researches directed toward a determination of the numbers of bacteria in soils from various sources, depths, conditions etc., and much valuable information was gained as to the relative numbers of organisms obtaining in soils under different conditions. But it came to be recognized after a few years that the method of enumeration of bacterial flora by plate counts falls far short of furnishing the needed data for a proper understanding of the functions of bacteria in soils.

Attention was then directed toward methods, not to determine the number of bacteria in soils, but to determine the abundance or efficiency of the particular kinds of organisms that are known to be either beneficial or injurious to agricultural soils. This problem was essayed in two different ways by Remy in 1902[2]), and Hiltner and Störmer in 1903[3]).

The method of Remy consists in using as an inoculum weighed amounts, usually one to ten grams, of the soil under investigation and with this inoculating sterile solutions of various composition to ascertain the chemical changes effected in the solutions by the bacteria introduced with the inoculum. In this way, for example, a 1 % peptone solution is inoculated, and the amount of ammonia found at the end of a given number of days is taken as an indication of the ammonifying power of the soil. Similarly, solutions containing ammonium compounds are employed to gain an index of the nitrifying power, and Giltay's solution is used to measure the denitrifying power.

The method of Hiltner and Störmer consists in using solutions similar to those employed in Remy's method, but making in each analysis a series of inoculations with the inoculum varying from 1000 milligrams to 0,001 milligram of soil. Since in some instances the dilution used fails to cause reaction because the quantity of inoculum is so small as to contain none of the specific organisms in question, while in other instances there is reaction, indicating the presence of the organism, some conclusions are permissible as to the number of bacteria present of the particular kind in question in the inoculum used.

The method of Remy and that of Hiltner and Störmer and modifications of these methods, have been used in numerous investigations in soil bacteriology, and much knowledge has been gained as

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1) The facts set forth in this article were presented in substantially this same form at the seventh annual meeting of the North Carolina Academy of Science May 2, 1908. (See Science. Vol. XXVII. p. 988.)

2) Centralbl. f. Bakt. Abt. II. Bd. VIII. 1902. p. 657, 699, 728 and 761.

3) Studien über die Bakterienflora des Ackerbodens. Berlin 1903. p. 474. (Reprinted from Arb. d. K. Gesundheitsamts. Biol. Abt. Bd. III. 1903.)

to the abilities of the bacteria of the soil to effect changes of particular kinds in the solutions employed and under the conditions of the tests.

The use of soil itself as a culture medium in which to study the activities of bacteria under conditions of bacteriological control seems to have been singularly neglected and when employed, has been used only for special purposes [c. f. L. C. Coleman[1]), and Bazarewski[2]), who employed soil in physiological studies of nitrifying organisms, and Wimmer[3]) and Hoffman[4])].

The object of soil bacteriology is to picture clearly the kinds and intensities of bacterial activity in the soil in order that the effects of cultural methods, fertilizers, crops etc., upon the bacterial flora and its efficiency may be known to the end that defects may be recognized and corrective measures employed.

That the methods of Remy, of Hiltner and Störmer and of their followers come much nearer to this ideal than mere colony enumeration, cannot be doubted, but that either of these methods very closely approximates actual occurrences in the soil itself is questionable. Conditions of bacterial life in solution and in soil may be, indeed, they certainly are, fundamentally different, and the most that can be said with assurance, when isolated species or natural or artificial composites of species, are inoculated into solutions of known composition and produce certain chemical changes, is that the organisms present bring about the changes noted under the conditions of the test, i. e., dealing with solutions of the artificial nature of those that are employed.

By such tests it has been noted that certain species of bacteria and certain live soils when placed in solution bring about nitrification, ammonification, denitrification etc. with certain degrees of intensity. It by no means follows of necessity that the same organisms when in soils. cause the same chemical changes, or if so, with the same intensities.

To picture adequately the functioning of soil bacteria, methods must be used which show as nearly as possible the effects as they are actually produced by bacteria in soils. To assume that bacterial changes produced in a solution represent the changes that would be caused by the same bacteria in a soil, is to base upon the hypothesis that the action of a given species of bacteria in a solution is the same in kind, and approximately the same in degree as is the action of the same species when in a soil.

Both upon a priori grounds and as the result of numerous observations during the past few years, we were lead to doubt the validity of this hypothesis[5]). A series of experiments was therefore instituted to test the question: "Do soil bacteria act in solutions the same as they do in soils". The results of these experiments, with other experiments of collateral bearing we here present and discuss.

We may state in advance that working with pure cultures as well

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1) Untersuchungen über Nitrifikation. (Centralbl. f. Bakt. Abt. II. Bd. XX. 1908. p. 401.)

2) Beiträge zur Kenntnis der Nitrifikation und Denitrifikation im Boden. [Diss.] Göttingen 1906.

3) Zeitschr. f. Hygiene. Bd. XLVIII. 1904. p. 160.

4) Relation of soil bacteria to nitrogenous decomposition. (23rd Rept. Wis. Agr. Exp. Sta. 1906. p. 120.)

5) Since the publication of the brief abstract of this paper in Science. Vol. XXVII. p. 988, Fraps, Bull. 106. Tex. Agr. Exp. Sta. p. 8, has noted that nitrification does not proceed in saturated soils and Lipman in his book "Bacteria in relation to country life", p. 338 says that "With too much (moisture) nitrification was suspended". Failure to nitrify in an almost "water logged" sand was also noted by Hoffman, 23rd Rept. Wis. Agr. Exp. Sta.

as with soil composites, there is a great divergence in behavior of the same organisms when in soils and when in solutions as regards nitrification[1], denitratation, and ammonification; that some species or races affect their chemical changes in each medium, some in one only, others to different degrees in different media.

## Bacteriological methods.

In all instances strict bacteriological precautions were taken to avoid contamination of the test cultures. In cases of composite or crude inoculations with soil, the soil to be used was taken with aseptic precautions and the water, media, glass ware etc. were sterile. Unless otherwise stated, the cultures were in cotton-stoppered, Erlenmeyer flasks, and were kept in a dark incubator room at a temperature varying from 30° to 35°. Calcium carbonate was added to all cultures, 400 grams (air dry weight) of soil were used with water sufficient to render one-third saturated, unless otherwise stated.

Before sterilizing, the soil and nitrogenous material (except asparagin) were mixed, the water content made as desired, and the whole flasked, plugged, autoclaved for 1 hour at 120° and inoculated as desired. To sterilize asparagin a definite amount was weighed into a graduated flask, and enough ether added to cover the asparagin. After twenty-four hours the ether was evaporated, sterile water added, the asparagin dissolved and made up to a definite volume. The desired amount of this solution was taken with a sterile pipette.

When it was desired to absorb solutions by soil, the soil to be used was dried in an air bath at 100—105°, then 200 or 400 grams of this soil were flasked, plugged, sterilized, and the solution to be absorbed, previously inoculated, was poured upon it.

Three methods of inoculation have been employed: 1st the desired quantity of fresh soil (the inoculum) was poured into the flask on top of the soil to be inoculated and mixed by thorough shaking. 2nd, the soil inoculum was weighed into a flask containing the nutrient solution and this mixture poured into the soil medium in the flask and mixed. 3rd, a bacterial suspension was secured by adding 100 grams of soil to 200 c. c. of sterile water and shaking three minutes. Inoculations were then made with the desired amount of the suspension.

## Chemical methods.

Soil extract. To the sample of 400 grams of soil were added 10 c. c. of chloroform and water sufficient with that originally in the soil to make 1200 c. c. Shaking in a shaking machine was continued for four hours. After standing over night, the solution was (1) poured off if sufficiently clear, or (2) filtered through a Pasteur-Chamberland filter[2], or (3) after an aliquot part had been taken for the ammonia determination, the remainder was treated with lime and carbon black and filtered after precipitating the lime with a current of carbon dioxide.

Ammonia. When nitrogen was added in the form of ammonium, sulphate, it was expelled by boiling with a solution of sodium hydroxide. When it was added in organic forms, it was expelled by boiling in presence of magnesium oxide. In either case the distillate was collected in one-fifth normal hydrochloric acid and titrated with one-tenth normal ammonium hydroxide solution using cochineal as an indicator.

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[1] Throughout this article we employ the term "nitrification" which has by many writers been used in many divers ways, in what seemed its most logical meaning, to designate the change of ammoniacal nitrogen to nitrate nitrogen, and the term nitritation and nitratation to mean respectively change to nitrite and nitrate, as has been done by Winogradsky. The term denitratation is introduced to designate change from the nitrate to some other form containing less oxygen.

[2] Bureau of soils U. S. Dept. Agr. Bull. No. 31. 1906. p. 12.

When the amount of nitrogen was less than the equivalent of 0,1 c. c. of one-tenth normal ammonium hydroxide solution, the ammonia was liberated and collected in the same manner but the amount was determined by Nessleriring.

Nitrites and nitrates. The clarified solution was tested with diphenylamine[1]) which shows as little as one part of nitrogen in the form of nitrite or nitrate in twenty millions or 0,05 milligrams nitrogen in one liter. In the case of soils where the dilution of the solution was approximately twenty times, the method would show 0,1 milligrams nitrogen in 100 c. c. of soil solution or about 0,06 milligrams in 400 grams of soil.

Nitrites. The sulphanilic acid method of Griess[2]) was used for nitrites.

Nitrates. Nitrates were usually determined by the Tiemann-Schulze method[3]) deduction being made for nitrites. When the amount of nitric oxide liberated was small, a correction was made for the amount insoluble in a concentrated solution of ferrous sulphate.

When the amount of nitrogen in the form of nitrates was very small, it was estimated by the phenol-disulphonic acid method[4]).

## Explanation of the Tables.

The amounts of ammonia, nitrites and nitrates are expressed in terms of the element nitrogen.

The amounts of nitrogen found at the end of the experiment in different forms of combinations are expressed in terms of percentages of the nitrogen added at the beginning of the experiment and in terms of milligrams in 100 c. c. of solution. In the case of soil, unless otherwise stated, the total amount of water present with 400 grams of soil was 60 c. c., which was sufficient for one-third saturation. In preparing the samples for experiment, the amount of water added was sufficient, together with that originally present in the soil, to make 60 c. c. approximately.

## Nitrification in soils and in solutions.

Experiment No. 32. 400 grams of soil No. 1866 from the bed of the greenhouse were placed in a litre Erlenmeyer flask, water containing ammonium sulphate to amount to 240 milligrams of nitrogen was added to make one-third saturation. Another flask was similarly prepared, except that the nitrogen was in the form of cottonseed meal. Checks, i. e., flasks similarly prepared, but sterile, were provided in each instance. The flasks were kept in the incubator room four weeks at 30—35°.

At the same time Omelianski's solution[5]) containing ammonium sulphate was inoculated with 5 grams of the same soil and maintained under the same conditions as were the soil cultures. The results appear in table 1.

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[1]) Treadwell Analytical Chemistry. Vol. I. 1906. p. 341.

[2]) Bul. Soc. Chim. Ser. 3. Vol. II. 1889. p. 347 or Bureau of Soils U. S. Dept. Agr. Bul. No. 31. 1906. p. 41.

[3]) Treadwell Anal. Chem. Vol. II. 1904. p. 360. — Zeitschr. f. anal. Chem. Bd. XIV. 1870. p. 401.

[4]) J. Am. Chem. Soc. 1896. p. 16, 122, 193 and Bureau of Soils U. S. Dept. Agr. Bul. No. 31. 1906. p. 39.

[5]) Centralbl. f. Bakt. Abt. II. Bd. V. p. 537—549.

**Table 1.** Showing nitrification of both ammonium sulphate and cottonseed meel in soils but not in solution.

Conditions of experiment 32.

| Sample No. | Date of inoculation | Days from inoculation to analysis | Medium |  | Inoculum |  | Initial nitrogen |  |
|---|---|---|---|---|---|---|---|---|
|  |  |  | Kind | Grams | Kind | Grams | Milligrams | Form |
| 1902 | 10/17/07 | 28 | Live soil 1866 | 400 | — | — | 240 | $(NH_4)_2SO_4$ |
| 1903 | „ | 28 | Sterile soil 1866 | 400 | — | — | 240 | $(NH_4)_2SO_4$ |
| 1904 | „ | 28 | Live soil 1866 | 400 | — | — | 240 | C. S. M. |
| 1905 | „ | 28 | Sterile soil 1866 | 400 | — | — | 240 | C. S. M. |
| 1905a | „ | 28 | Omelianskis solution | 300 | Soil 1866 | 5 | 127,3 | $(NH_4)_2SO_4$ |

Results of analysis. Experiment 32.

| No. | Percentage of initial nitrogen |  |  |  |  | Milligrams of nitrogen in 100 cc. solut. |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
|  | Unaccounted form | Total recovered | Recovered as |  |  | Unaccounted form | Total recovered | Recovered as |  |  |
|  |  |  | Ammonia | Nitrites | Nitrates |  |  | Ammonia | Nitrites | Nitrates |
| 1902 | 10,00 | 90,00 | 34,00 | tr. | 56,00 | 40,0 | 360,0 | 136,0 | tr. | 224,0 |
| 1903 | 12,40 | 87,60 | 82,00 | 0,20 | 5,60 | 49,6 | 350,4 | 328,0 | 0,8 | 22,4 |
| net | — | — | — | 0,00 | 50,40 | — | — | — | 0,0 | 201,6 |
| 1904 | 54,00 | 46,00 | 0,00 | tr. | 46,00 | 216,0 | 184,0 | 0,0 | 0,0 | 184,0 |
| 1905 | 100,00 | — | — | 0,00 | 0,00 | 400,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| net | — | — | — | — | 46,00 | — | — | — | 0,0 | 184,0 |
| 1902a | — | — | 0,00 | — | — | — | — | 0,0 | — | — |

C. S. M. = cottonseed meal.

It is here evident from the soil tests both with ammonium sulphate and with cottonseed meal that this soil is in vigorous nitrifying condition, yet the tests by inoculation into solution give no indication of the presence of nitrate, even by diphenylamine capable of indicating one part in two millions of nitrate. It may be added that this soil has never nitrified in solution sufficiently to give the diphenylamine reaction, though in soil tests it has repeatedly showed itself to be in reality a vigorous nitrifier.

The entire absence of ammonia in the cultures with cottonseed meal was so striking and unexpected that the samples were again analyzed, Dec. 5th, with confirmation of the fact that neither of the cottonseed meal flasks contained ammonia.

This raised the question whether the soil under study had any ammonifying power and tests were made, employing both peptone and cottonseed meal separately and inoculating with soil 1866. Each when tested at the end of eight days gave a considerable amount of ammonia, showing the soil to be in normal ammonifying condition. The only probable explanation of the absence of ammonia in this instance is that it was consumed by the nitrite organisms as fast as it was made. It appears also that the nitrite was likewise consumed by the nitrate organisms equally fast.

Experiment No. 34. Three samples of soils No. 1667, 1867 and plat 10, 400 grams each, were taken. The soil was made to one-third saturation with water carrying 240 milligrams of nitrogen either as ammonium sulphate or as cottonseed meal. Simultaneous inoculations were made into Omelianski's ammonia solution, using 5 grams of soil as the inoculum. All were done in duplicate. The results are given in table 2.

Table 2. Showing nitrification of both ammonium sulphate and cottonseed meal by soil No. 1867 in soil but not in solution.
Conditions of experiment 34.

| Sample No. | Date of inoculation | Time of incubation days | Medium | | Inoculum | | Initial nitrogen | |
|---|---|---|---|---|---|---|---|---|
| | | | Kind | Grams | Kind | Grams | Milligrams | Form. |
| 1909 | 10/30/07 | 28 | Live soil 1667 | 400 | — | — | 240 | $(NH_4)_2SO_4$ |
| 1910 | „ | 28 | „ „ 1667 | 400 | — | — | 240 | C. S. M. |
| 1911 | „ | 28 | „ „ plat 10 | 400 | — | — | 240 | $(NH_4)_2SO_4$ |
| 1912 | „ | 28 | „ „ plat 10 | 400 | — | — | 240 | C. S. M. |
| 1913 | „ | 28 | „ „ 1867 | 400 | — | — | 240 | $(NH_4)_2SO_4$ |
| 1914 | „ | 28 | „ „ 1867 | 400 | — | — | 240 | C. S. M. |
| 1909a | „ | 28 | Omelianski's solut. | 300 | Soil 1667 | 5 | 127,3 | $(NH_4)_2SO_4$ |
| 1911a | „ | 28 | „ „ | 300 | Soil plat 10 | 5 | 127,3 | $(NH_4)_2SO_4$ |
| 1913a | „ | 28 | „ „ | 300 | Soil 1867 | 5 | 127,3 | $(NH_4)_2SO_4$ |

Results of analysis. Experiment 34.

| No. | Percentage of initial nitrogen | | | | | Milligrams nitrogen in 100 c. c. solut. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unaccounted form | Total recovered | Recovered as | | | Unaccounted form | Total recovered | Recovered as | | |
| | | | Ammonia | Nitrites | Nitrates | | | Ammonia | Nitrites | Nitrates |
| 1909 | 12,20 | 87,80 | 87,80 | tr. | 0,00 | 73,2 | 526,8 | 526,8 | tr. | 0,0 |
| 1910 | 81,50 | 18,50 | 18,50 | „ | 0,00 | 489,0 | 111,0 | 111,0 | „ | 0,0 |
| 1911 | 20,70 | 79,30 | 79,30 | „ | 0,00 | 124,2 | 475,8 | 475,8 | „ | 0,0 |
| 1912 | 82,40 | 17,60 | 17,60 | „ | 0,00 | 494,4 | 105,6 | 105,6 | „ | 0,0 |
| 1913 | 1,70 | 98,30 | 42,10 | „ | 56,20 | 6,8 | 393,2 | 168,4 | „ | 224,8 |
| 1914 | 7,30 | 92,30 | 42,10 | „ | 50,20 | 29,2 | 370,8 | 168,4 | „ | 200,8 |
| 1909a | — | — | 0,00 | „ | 0,00 | — | — | 0,0 | — | — |
| 1911a | — | — | 0,00 | „ | 0,00 | — | — | 0,0 | — | — |
| 1913a | — | — | 0,00 | „ | 0,00 | — | — | 0,0 | — | — |

C. S. M. = cottonseed meal.

It is noted in this instance two of the three samples failed utterly to nitrify either in soil or in solution, but that one sample, soil No. 1867, nitrified strongly in soil though not at all in solution. This soil itself was known to be nearly free from nitrates.

Experiment No. 108. Two soils were inoculated in duplicate into soils and at the same time in duplicate into Omelianski's solution. The results in table 3 give another instance of failure to nitrify in solutions though nitrification in soil was vigorous. Though no checks were run, it is known that soil 193, contained practically no nitrate and that the nitrate shown in this table is the result of nitrification during the experiment.

(See Table 3 p. 361.)

Experiment No. 72. Cultures were tested by using soil extract as a medium, to ascertain whether some deficiency in the chemical constitution of the solutions used, which might not exist in soil extract itself, was responsible for the failure to nitrify in solutions.

Tests were alse made in Wiley's solution[1]) and Omelianski's solution using 10 grams of inoculum.

The results at the end of four weeks are given in table 4.

(See Table 4 p. 361.)

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1) Wiley, Yearbook of U. S. Dept. Agr. 1895. p. 97.

Table 3. Showing failure to nitrify in solution but vigorous nitrification in soils. Conditions of experiment 108.

| Sample No. | Date of inoculation | Time of incubation days | Medium |  | Inoculum |  | Initial nitrogen |  |
|---|---|---|---|---|---|---|---|---|
|  |  |  | Kind | Grams | Kind | Grams | Milligrams | Form. |
| 2546 | 8/24/08 | 28 | Live soil 1931a | 400 | — | — | 240 | $(NH_4)_2SO_4$ |
| 2547 | „ | 28 | Live soil 1931a | 400 | — | — | 240 | „ |
| 2548 | „ | 28 | Omelianski's solut. | 377,12 | Suspension of soil 1931a | 10 | 160 | „ |
| 2549 | „ | 28 | Omelianski's solut. | 377,12 | Suspension of soil 1931a | 10 | 160 | „ |
| 2550 | „ | 28 | Live soil 1931b | 400 | — | — | 240 | „ |
| 2551 | „ | 28 | Live soil 1931b | 400 | — | — | 240 | „ |
| 2552 | „ | 28 | Omelianski's solut. | 377,12 | Suspension of soil 1931b | 10 | 160 | „ |
| 2553 | „ | 28 | Omelianski's solut. | 377,12 | Suspension of soil 1931b | 10 | 160 | „ |

Results of analysis. Experiment 108.

| No. | Percentage of initial nitrogen |  |  |  |  | Milligrams nitrogen in 100 c. c. solut. |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
|  | Unaccounted form | Total recovered | Recovered as |  |  | Unaccounted form | Total recovered | Recovered as |  |  |
|  |  |  | Ammonia | Nitrites | Nitrates |  |  | Ammonia | Nitrites | Nitrates |
| 2546 | — | — | 66,52 | 0,34 | 3,38 | — | — | 133,0 | 0,7 | 6,8 |
| 2547 | — | — | 67,78 | 1,50 | 8,45 | — | — | 135,6 | 3,0 | 16,9 |
| aver. | 26,93 | 73,07 | 67,15 | 0,92 | 5,92 | 53,9 | 146,1 | 134,3 | 1,8 | 11,8 |
| 2548 | — | — | 93,78 | 0,00 | 0,00 | — | — | 39,8 | 0,0 | 0,0 |
| 2549 | — | — | 96,36 | 0,00 | 0,00 | — | — | 40,9 | 0,0 | 0,0 |
| aver. | 4,93 | 95,07 | 95,07 | 0,00 | 0,00 | 2,1 | 40,4 | 40,4 | 0,0 | 0,0 |
| 2550 | — | — | 53,91 | 0,85 | 33,20 | — | — | 107,8 | 1,6 | 66,4 |
| 2551 | — | — | 62,46 | 0,88 | 7,39 | — | — | 124,9 | 1,8 | 14,6 |
| aver. | 21,68 | 78,32 | 58,18 | 0,87 | 20,24 | 42,7 | 157,3 | 116,3 | 1,7 | 41,0 |
| 2552 | — | — | 93,60 | 0,00 | 0,00 | — | — | 39,8 | 0,0 | 0,0 |
| 2553 | — | — | 94,38 | 0,00 | 0,00 | — | — | 40,1 | 0,0 | 0,0 |
| aver. | 6,12 | 93,98 | 93,98 | 0,00 | 0,00 | 2,6 | 39,9 | 39,9 | 0,0 | 0,0 |

Table 4. Showing better nitrification in soil than in soil extract or in the usual culture solutions. Conditions of experiment 72.

| Sample No. | Date of inoculalation | Days from inoculation to analysis | Medium |  | Inoculum |  | Initial nitrogen |  |
|---|---|---|---|---|---|---|---|---|
|  |  |  | Kind | Grams | Kind | Grams | Milligrams | Form. |
| 2046 | 2/10/08 | 28 | Sterile soil 1867 | 390 | Soil 1867 | 10 | 240 | $(NH_4)_2SO_4$ |
| 2047 | „ | 28 | Sterile soil 1867 | 390 | „ 1867 | 10 | 240 | „ |
| 2048 | „ | 28 | Omelianski's solut. | 300 | „ 1867 | 10 | 127,3 | „ |
| 2049 | „ | 28 | Wiley's solution | 300 | „ 1867 | 10 | 12,73 | „ |
| 2050 | „ | 28 | Extract of soil 1867 | 300 | „ 1867 | 10 | 127,3 | „ |
| 2054 | „ | 28 | Omelianski's solut. | 300 | „ 1867 | 0,2 | 127,3 | „ |
| 2055 | „ | 28 | Wiley's solution | 300 | „ 1867 | 0,2 | 12,73 | „ |

Results of analysis. Experiment 72.

| No. | Percentage of initial nitrogen | | | | | Milligrams nitrogen in 100 c. c. solut. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unaccounted form | Total recovered | Recovered as | | | Unaccounted form | Total recovered | Recovered as | | |
| | | | Ammonia | Nitrites | Nitrates | | | Ammonia | Nitrites | Nitrates |
| 2046 | 19,00 | 81,00 | 59,50 | 0,10 | 21,50 | 76,0 | 324,0 | 238,0 | 0,0 | 86,0 |
| 2047 | 10,00 | 90,00 | 64,40 | 0,00 | 25,60 | 40,0 | 360,0 | 257,6 | 0,0 | 102,4 |
| aver. | 14,50 | 85,50 | 61,95 | 0,05 | 23,55 | 58,0 | 342,0 | 247,8 | 0,0 | 94,2 |
| 2048 | 1,20 | 98,80 | 97,00 | 0,00 | 1,80 | 0,5 | 42,0 | 41,2 | 0,0 | 0,8 |
| 2049 | 12,90 | 87,10 | 42,90 | 0,00 | 44,20[2] | 0,6 | 3,7 | 1,8 | 0,0 | 1,9 |
| 2050 | 5,70 | 94,30 | 81,20 | 0,10 | 13,10 | 2,4 | 40,1 | 34,5 | tr. | 5,6 |
| 2054 | 12,20 | 87,80 | 87,10 | 0,00 | 0,70 | 5,5 | 37,0 | 37,0 | 0,0 | tr. |
| 2055 | 9,70[1] | 109,70 | 102,30 | 0,00 | 7,40[2] | 0,4[1] | 4,7 | 4,4 | 0,0 | 0,3 |

The contrast is here again strong between soil cultures Nos. 2046, 2047, which either upon a basis of percentage of nitrification or upon a basis of milligrams nitrified per c. c. of solution show a much greater nitrification than culture in Omeliansky's solution (Nos. 2048 and 2054), which practically failed to nitrify at all. While the percentage of nitrification in Nos. 2049 and 2055 in Wiley's solution is high, this is not significant since the absolute amount of nitrates is so small as to be almost negligible, c. f. initial amounts of nitrogen present. A more nearly correct comparison regarding the nitrifying power of these numbers is shown in the last column with only slight nitrification in the liquid medium. While there was nitrification in the soil extract No. 2050, it fell far short of that occurring in the soil itself. While this experiment taken alone would tend to indicate that soil extract contains something favorable to nitrification which is lacking in the artificial solutions, this conclusion is not supported by other evidence. The conclusion that soil as a medium furnishes a much more sensitive means for detection of nitrification than do the usual solutions, is however supported by the evidence throughout.

Experiment No. 72 (later.), Duplicate cultures of this experiment were analyzed again at the end of 64 days. During this additional time some slight nitrification had occurred in the cultures which showed no nitrification at first analysis, but the superiority of soil as a medium for nitrification is even more apparent at the second than at the first analysis, c. f. Tables 4 and 5 last column.

(See Table 5 p. 363.)

Basing nitrification upon the amount of nitrate produced per 100 c. c. of solution, it is seen that the process is 68 times as rapid in the soil water in situ as it is in Omelianski's or Wiley's solutions and 39 times as fast as it is in an extract of the same soil.

It seems clear from this experiment that not only are results of four week tests in solutions such as those of Wiley, Omelianski or Ashby worthless as indicative of the nitrifying powers of soils tested by them, but that results which are negative at the end of four weeks may indicate some nitrification if the cultures be allowed to stand for longer time.

Experiment No. 73: To 400 grams each of live soils Nos. 1867,

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[1] Excess.

[2] This percentage has slight significance because of the small amount of initial nitrogen.

Table 5. Showing results of nitrification tests in solutions and in soils at 64 days.
Conditions of experiments 72.

| Sample No. | Date of inoculation | Days of incubation | Medium | | Inoculum | | Initial nitrogen | |
|---|---|---|---|---|---|---|---|---|
| | | | Kind | Grams | Kind | Grams | Milligrams | Form. |
| 2165 | 2/10/08 | 64 | Sterile soil 1867 | 390 | soil 1867 | 10 | 240 | $(NH_4)_2SO_4$ |
| 2166 | „ | 64 | Sterile soil 1867 | 390 | „ | 10 | 240 | „ |
| aver. | | | | | | | | „ |
| 2167 | „ | 64 | Omelianski's solution | 300 | „ | 10 | 127.3 | „ |
| 2168 | „ | 64 | Wiley's solution | 300 | „ | 10 | 12,73 | „ |
| 2169 | „ | 64 | Extract of soil 1867 | 300 | „ | 10 | 127,3 | „ |
| 2173 | „ | 64 | Omelianski's solution | 300 | „ | 0,2 | 127,3 | „ |
| 2174 | „ | 64 | Wiley's solution | 300 | „ | 0,2 | 12,73 | „ |

Results of analysis. Experiment 72.

| No. | Percentage of initial nitrogen | | | | | Milligrams nitrogen in 100 c. c. solut. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unaccounted form | Total recovered | Recovered as | | | Unaccounted form | Total recovered | Recovered as | | |
| | | | Ammonia | Nitrites | Nitrates | | | Ammonia | Nitrites | Nitrates |
| 2165 | 6,80 1) | 106,80 | 30,80 | 0,00 | 76,00 | 27,2 1) | 427,2 | 123,2 | 0,0 | 304,0 |
| 2166 | 1,20 1) | 101,20 | 23,80 | 0,00 | 77,40 | 4,8 1) | 404,8 | 95,2 | 0,0 | 309,6 |
| aver. | 4,00 1) | 104,00 | 27,30 | 0,00 | 76,70 | 16,0 1) | 416,0 | 109,2 | 0,0 | 306,8 |
| 2167 | 3,00 1) | 103,00 | 92,40 | 0,00 | 10,60 | 1,3 1) | 43,8 | 39,3 | 0,0 | 4,5 |
| 2168 | 3,40 | 96,60 | 6,60 | 0,00 | 90,00 2) | 0,2 | 4,1 | 0,3 | 0,0 | 3,8 |
| 2169 | 4,70 | 95,30 | 77,20 | 0,00 | 18,10 | 2,0 | 40,5 | 32,8 | 0,0 | 7,7 |
| 2173 | 6,90 1) | 106,90 | 97,70 | 1,30 | 9,20 | 2,8 1) | 45,3 | 41,4 | 0,6 | 3,9 |
| 2174 | 1,60 1) | 101,60 | 26,40 | tr. | 75,20 2) | 0,1 1) | 4,3 | 1,1 | tr. | 3,2 |

1667, 2069 were added in the usual way 240 milligrams of nitrogen as cottonseed meal.

Flasks of Omelianski's and of Wiley's solutions were also inoculated with each of these soils. Determinations were made upon all after 4 weeks' incubation.

The results are presented in table 6.

Table 6. Showing nitrification in soils, not in sulutions.
Conditions of experiment 73.

| Sample No. | Date of inoculation | Time incubation days | Medium | | Inoculum | | Initial nitrogen | |
|---|---|---|---|---|---|---|---|---|
| | | | Kind | Grams | Kind | Grams | Milligrams | Form |
| 2060 | 2/13/08 | 28 | Live soil 1867 | 400 | — | — | 240 | C. S. M. 3) |
| 2057 | „ | 28 | Sterile soil 1867 | 400 | — | — | 0,12 | „ |
| 2061 | „ | 28 | Live soil 1667 | 400 | — | — | 240 | „ |
| 2058 | „ | 28 | Sterile soil 1667 | 400 | — | — | 0,12 | „ |
| 2062 | „ | 28 | Live soil 2069 | 400 | — | — | 240 | „ |
| 2059 | „ | 28 | Sterile soil 2069 | 400 | — | — | 0,12 | „ |
| 2063 | „ | 28 | Wiley's solution | 300 | Soil 1867 | 0,2 | 12,7 | $(NH_4)_2SO_4$ |
| 2064 | „ | 28 | „ „ | 300 | „ 1667 | 0,2 | 12,7 | „ |
| 2065 | „ | 28 | „ „ | 300 | „ 2069 | 0,2 | 12,7 | „ |
| 2066 | „ | 28 | Omelianski's solution | 300 | „ 1867 | 0,2 | 127 | „ |
| 2067 | „ | 28 | „ „ | 300 | „ 1667 | 0,2 | 127 | „ |
| 2068 | „ | 28 | „ „ | 300 | „ 2069 | 0,2 | 127 | „ |

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1) Excess.

2) These percentages have small significance because of the small amount of nitrogen.

3) C. S. M. = cottonseed meal.

Results of analysis. Experiment 73.

| No. | Percentage of initial nitrogen | | | | | Milligrams nitrogen in 100 c. c. sol. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unaccounted form | Total recovered | Recovered as | | | Unaccounted form | Total recovered | Recovered as | | |
| | | | Ammonia | Nitrites | Nitrates | | | Ammonia | Nitrites | Nitrates |
| 2060 | — | — | — | — | — | 155,6 | 244,4 | 14,0 | 0,0 | 230,4 |
| 2057 | — | — | — | — | — | 0,4 | 2,4 | 2,4 | 0,0 | 1,6 |
| net | 39,90 | 60,10 | 2,90 | 0,00 | 57,20 | 158,0 | 242,0 | 11,6 | 0,0 | 228,8 |
| 2061 | — | — | — | — | — | 318,0 | 82,0 | 78,4 | 0,0 | 3,6 |
| 2058 | — | — | — | — | — | 1,0 | 1,0 | 0,6 | 0,0 | 0,4 |
| net | 79,75 | 20,25 | 19,45 | 0,00 | 0,80 | 319,0 | 81,0 | 77,8 | 0,0 | 3,2 |
| 2062 | — | — | — | — | — | 260,0 | 140,0 | 33,6 | 6,4 | 106,4 |
| 2059 | — | — | — | — | — | 1,3 | 0,7 | 0,3 | 0,0 | 0,4 |
| net | 65,18 | 34,82 | 8,32 | 1,60 | 24,90 | 260,7 | 139,3 | 33,3 | 6,4 | 99,6 |
| 2063 | 41,00 | 59,00 | 0,00 | 3,60 | 59.00 | 1,8 | 2,5 | 0,0 | 0,2 | 2,5 |
| 2064 | 41,90 | 58,10 | 33,00 | 0,00 | 25,10 | 1,8 | 2,5 | 1,4 | 0,0 | 1,1 |
| 2065 | 50,10 | 49,90 | 39,60 | tr. | 10,30[1] | 2,2 | 2,1 | 1,7 | tr. | 0,4 |
| 2066 | 55,10 | 44,90 | 40,90 | 0,20 | 4,00 | 23,4 | 19,1 | 17,4 | „ | 1,7 |
| 2067 | 61,40 | 38,60 | 37,60 | tr. | 1,00 | 26,1 | 16,4 | 16,0 | „ | 0,4 |
| 2068 | 50,90 | 49,10 | 48,10 | „ | 11,00 | 21,7 | 20,8 | 20,4 | „ | 0,4 |

In the last column of the table it is noted that the soils 1867 and 2069 gave a large nitrification in soil but a nearly negligible qantity in Omelianski's or Wiley's solutions, while the third soil failed to nitrify to any extent in any medium.

Making proper allowance for the very small quantity of initial nitrogen in Omelianski's and Wiley's solutions, the same conclusion is reached from the percentage of nitrogen recovered as nitrates. Two of the soils could nitrify vigorously in soils but only sligthly in solutions.

Experiment No. 69a. Four cultures were made; two in soils, one with peptone and one with cottonseed meal; two in solutions, one with peptone and one with cottonseed meal. They were inoculated in parallel with the same bacterial suspensions from soil No. 1867. Analysis on the 14th day gave the results shown in table 7.

Table 7. Showing nitrification in 14 days in soils, not in solutions.
Conditions of experiment 69a.

| Sample No. | Date of inoculation | Time of incubation days | Medium | | Inoculum | | Initial nitrogen | |
|---|---|---|---|---|---|---|---|---|
| | | | Kind | Grams | Kind | Grams | Milligrams | Form. |
| 2025 | 2/10 08 | 14 | Sterile soil 1867 | 490 | Soil 1867 | 10 | 120 | Peptone |
| 2026 | „ | 14 | „ „ 1867 | 490 | „ 1867 | 10 | 120 | C. S. M. |
| 2027 | „ | 14 | Peptone water | 200 | „ 1867 | 10 | 60 | Peptone |
| 2028 | „ | 14 | C. S. M. water | 200 | „ 1867 | 10 | 60 | C. S. M. |

Results of analysis. Experiment 69a.

| No. | Percentage of initial nitrogen | | | | | Milligrams nitrogen in 100 c. c. solut. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unaccounted form | Total recovered | Recovered as | | | Unaccounted form | Total recovered | Recovered as | | |
| | | | Ammonia | Nitrites | Nitrates | | | Ammonia | Nitrites | Nitrates |
| 2025 | 51,60 | 48,40 | 11,20 | 1,50 | 37,20 | 103,2 | 96,8 | 22,4 | 3,0 | 74,4 |
| 2026 | 66,30 | 33,70 | 2,80 | 1,50 | 30,90 | 132,6 | 67,4 | 5,6 | 3,0 | 61,8 |
| 2027 | 29,60 | 70,40 | 70,40 | 0,00 | 0,00 | 8,9 | 21,1 | 21,1 | 0,0 | 0,0 |
| 2028 | 61,70 | 38,30 | 28,30 | 0,00 | 0,00 | 18,5 | 11,5 | 11,5 | 0,0 | 0,0 |

C. S. M. = cottonseed meal.

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[1] Not significant on account of the very small amount of initial nitrogen.

While nitrification progressed to marked extent in both soil cultures, the solutions gave no reaction with diphenylamine. Though no checks were run with this test to determine the amounts of nitrates in these soils, our constant experimentation with them and frequent analysis assure us that there was barely a trace, or no nitrate at all, present in the soil when the experiment was set up. The amount shown by the analyses is due to nitrification during the experiment.

Experiments 56 and 57. It seemed possible that the amount of inoculum used might be an important factor in determining the amount, or at least the rapidity, of nitrification in early stages of incubation. To throw light upon this point, parallel cultures were made by mixing the same soil, sterile and alive, in different proportions, thus varying practically only one factor, the quantity of live organism present. Experiment 56 consisted of such a series with nitrogen supplied in the form of ammonium sulphate; experiment 57, with nitrogen in the form of cottonseed meal.

The results are given in the tables 8 and 9.

Table 8. Showing influence of quantity of bacteria upon rapidity of nitrification.
Conditions of experiment 56.

| Sample No. | Date of inoculation | Time of incubation days | Medium | | Inoculum | | Initial nitrogen | |
|---|---|---|---|---|---|---|---|---|
| | | | Kind | Grams | Kind | Grams | Milligrams | Form. |
| 1962 | 12/2/08 | 28 | Sterile soil 1867 | 390 | soil 1867 | 10 | 240 | $(NH_4)_2SO_4$ |
| 1963 | „ | 28 | „ „ 1867 | 350 | „ 1867 | 50 | 240 | „ |
| 1964 | „ | 28 | „ „ 1867 | 300 | „ 1867 | 100 | 240 | „ |
| 1965 | „ | 28 | „ „ 1867 | 200 | „ 1867 | 200 | 240 | „ |
| 1966 | „ | 28 | Live soil 1867 | 400 | „ — | — | 240 | „ |
| 1967 | „ | 28 | Sterile soil 1867 | 400 | „ — | — | 240 | „ |

Results of analysis. Experiment 56.

| No. | Percentage of initial nitrogen | | | | | Milligrams nitrogen in 100 c. c. solut. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unaccounted form | Total recovered | Recovered as | | | Unaccounted form | Total recovered | Recovered as | | |
| | | | Ammonia | Nitrites | Nitrates | | | Ammonia | Nitrites | Nitrates |
| 1962 | — | — | 60,90 | 0,20 | lost | — | — | 243,6 | 0,8 | lost |
| 1963 | 29,90 | 71,10 | 53,20 | 0,20 | 17,90 | 115,6 | 284,6 | 212,8 | 0,8 | 71,6 |
| 1964 | 34,20 | 65,80 | 42,70 | 0,20 | 23,10 | 136,8 | 263,2 | 170,8 | 0,8 | 97,4 |
| 1965 | 21,40 | 78,60 | 44,10 | 0,20 | 34,50 | 85,6 | 314,4 | 176,4 | 0,8 | 138,0 |
| 1966 | 4,60[1] | 104,60 | 12,60 | 0,20 | 91,80 | 17,6[1] | 417,6 | 50,4 | 0,8 | 367,2 |
| 1967 | 31,20 | 68,60 | 68,60 | 0,20 | 0,00 | 124,8 | 275,2 | 274,4 | 0,8 | 0,0 |

Table 9. Showing influence of quantity of bacteria upon rapidity of nitrification of cottonseed meal.
Conditions of experiment 57.

| Sample No. | Date of inoculation | Time of incubation days | Medium | | Inoculum | | Initial nitrogen | |
|---|---|---|---|---|---|---|---|---|
| | | | Kind | Grams | Kind | Grams | Milligrams | Form |
| 1968 | 12/5/08 | 28 | Sterile soil 1867 | 390 | Soil 1867 | 10 | 240 | C. S. M. |
| 1969 | „ | 28 | „ „ 1867 | 350 | „ 1867 | 50 | 240 | „ |
| 1970 | „ | 28 | „ „ 1867 | 300 | „ 1867 | 100 | 240 | „ |
| 1971 | „ | 28 | „ „ 1867 | 200 | „ 1867 | 200 | 240 | „ |
| 1972 | „ | 28 | Live soil 1867 | 400 | — | — | 240 | „ |
| 1973 | , | 28 | Sterile soil 1867 | 400 | — | — | 240 | „ |

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1) Excess.

Results of analysis. Experiment 57.

| No. | Percentage of initial nitrogen | | | | | Milligrams nitrogen in 100 c.c. solut. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unaccounted form | Total recovered | Recovered as | | | Unaccounted form | Total recovered | Recovered as | | |
| | | | Ammonia | Nitrites | Nitrates | | | Ammonia | Nitrites | Nitrates |
| 1968 | 76,20 | 23,80 | 17,50 | tr. | 6,30 | 304,8 | 95,2 | 70,0 | tr. | 25,2 |
| 1969 | 70,10 | 29,90 | 13,30 | „ | 16,60 | 280,4 | 119,6 | 53,2 | „ | 66,4 |
| 1970 | 74,50 | 25,50 | 22,40 | „ | 3,10 | 298,0 | 102,0 | 89,6 | „ | 12,4 |
| 1971 | 60,20 | 39,80 | 4,90 | „ | 34,90 | 240,8 | 159,2 | 19,6 | „ | 139,6 |
| 1972 | 53,60 | 47,00 | 0,00 | „ | 47,00 | 212,0 | 188,0 | 0,0 | „ | 188,0 |
| 1973 | 97,50 | 2,50 | 0,00 | „ | 2,50 | 390,0 | 10,0 | 0,0 | „ | 10,0 |

C. S. M. = cottonseed meal.

In table 8 there is shown a remarkably regular increase in nitrification as the quantity if inoculum increases. In table 9 where cottonseed meal was used, if the third number be disregarded, the same relation holds true. The descrepancy in the behavior of No. 1970 is inexplicable, but it probably does not vitiate the general conclusions supported by these two experiments, viz., that with increase in the inoculum there is nearly a corresponding increase in the total amount of nitrification at the end of four weeks.

When tests of nitrifying power are made in the soil itself by adding the material to be nitrified directly to the soil, it is evident that there is initially a much larger number of organisms than when 200 milligrams or even 10 grams of the soil are used as an inoculum. We may regard the direct soil tests in our work as having the equivalent of an inoculum of 400 grams.

The idea that increase in the quantity of the inoculum brings increase in nitrification arouses the suspicion that perhaps the low nitrification, or entire failure to nitrify, found by the Ashby1) method with an inoculum of only 200 milligrams of soil, may be due to the smallness of the inoculum used.

Experiment No. 58 was set up to test this point: to determine whether the failure to nitrify in solutions can fairly be attributed to the smallness of the inoculum used.

A series of tests was made in solutions varying the amount of inoculum. In order to maintain conditions as nearly parallel as possible, the quantity of soil used in each case was the same, a larger or smaller amounts of it being sterilized to give the desired variation in the number of live organisms present.

Thus in No. 1974, 399,5 grams ofs terile soil + 0,5 grams of the same live soil were used, while in No. 1979, 0 grams of sterile soil + 400 grams of live soil were used. The other numbers gave intermediate conditions. Solutions sufficient to cover the soil to a depth of 2,5 cm was employed in each case, the solution also carrying nitrogen at the rate of 240 milligrams per 400 c. c. The culture was frequently shaken, sampled, and tested by diphenylamine with negative results. At the end of four weeks, analysis was also made with still no nitrification, as is shown in table 10.

(See Table 10 p. 367.)

A comparison of these results with those of experiments 56 and 57 which were inoculated with the same soils, brings out again clearly the

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1) Journ. Chem. Soc. Vol. LXXXXV. p. 1158.

Table 10. Showing no nitrification in solutions even when very large inoculum was used.

Conditions of experiment 58.

| Sample No. | Date of inoculation | Time of incubation days | Medium | | Inoculum | | Initial nitrogen | |
|---|---|---|---|---|---|---|---|---|
| | | | Kind | Grams | Kind | Grams | Milligrams | Form |
| 1974 | 12/12/07 | 28 | Sterile soil 1867<br>Water | 399,5<br>200 | Soil 1867 | 0,5 | 360 | C. S. M. |
| 1975 | „ | 28 | Sterile soil 1867<br>Water | 300<br>200 | „ 1867 | 100 | 360 | „ |
| 1976 | „ | 28 | Live soil 1867<br>Water | 400<br>200 | | — | 360 | „ |
| 1977 | „ | 28 | Sterile soil 1867<br>Water | 399,5<br>200 | „ 1867 | 0,5 | 360 | $(NH_4)_2SO_4$ |
| 1978 | „ | 28 | Sterile soil 1867<br>Water | 300<br>200 | „ 1867 | 100 | 360 | „ |
| 1979 | „ | 28 | Live soil 1867<br>Water | 400<br>200 | | — | 360 | „ |

Results of analysis. Experiment 58.

| No. | Percentage of initial nitrogen | | | | | Miligrams nitrogen in 100 c. c. solut. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unaccounted form | Total recovered | Recovered as | | | Unaccounted form | Total recovered | Recovered as | | |
| | | | Ammonia | Nitrites | Nitrates | | | Ammonia | Nitrites | Nitrates |
| 1974 | 70,60 | 29,40 | 29,40 | 0,00 | 0,00 | 97,8 | 40,7 | 40,7 | 0,0 | 0,0 |
| 1975 | 48,90 | 51,10 | 51,10 | tr. | 0,00 | 67,7 | 70,8 | 70,8 | tr. | 0,0 |
| 1976 | 78,30 | 21,70 | 21,70 | 0,00 | 0,00 | 108,4 | 30,1 | 30,1 | 0,0 | 0,0 |
| 1977 | 45,40 | 54,60 | 54,60 | 0,00 | 0,00 | 62,9 | 75,6 | 75,6 | 0,0 | 0,0 |
| 1978 | lost | lost | lost | lost | 0,00 | lost | lost | lost | lost | 0,0 |
| 1979 | 43,20 | 56,80 | 56,70 | tr. | 0,00 | 60,0 | 78,5 | 78,5 | tr. | 0,0 |

C. S. M. = cottonseed meal.

failure of the soil to nitrify at all in the solutions, although the same soil can nitrify vigorously in the same period of time, as is shown by actual soil tests.

Experiment No. 58 demonstrates conclusively that the differences existing between results of cultures in soils and in solutions are not to be explained by the differences in the quantities of inoculum used.

Experiment 211. It being demonstrated that the water relation is a very important one as regards nitrification, and that nitrification proceeds not at all, or very slightly in solutions, during the time of our experiments with the soils under consideration, but vigorously in soil itself, it became desirable to determine whether nitrification can proceed in a saturated soil as well as in soils of less water content. To this end cultures in three different soils were prepared, some bearing water to $^1/_3$, $^2/_3$, and some to complete saturation. The cultures were inoculated with a suspension of soil 1867.

The results are given in table 11.

Table 11. Showing failure to nitrify in saturated soils. Nitrogen 240 mgs. as $(NH_4)_2SO_4$.

Conditions of experiment 211. Results of analysis. Experiment 211.

| Sample No. | Date of inoculation | Days from inoculation to analysis | Medium | | Inoculum | | No. | Percentage of initial nitrogen | | | | | Milligrams nitrogen in 100 c. c. sol. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Kind | Amount | Kind | Amount | | Unaccounted form | Total recovered | Recovered as | | | Unaccounted form | Total recovered | Recovered as | | |
| | | | | | | | | | | Ammonia | Nitrites | Nitrates | | | Ammonia | Nitrites | Nitrates |
| 2621 | 9/10/08 | 28 | Sterile soil 1549<br>Water to ²/₃ saturation | 400<br>60 | Suspension of soil No. 1867 | 10 c. c. | 2621 | | | 24,51 | 2,25 | 24,29 | | | 98,0 | 9,0 | 97,2 |
| 2622 | „ | 28 | Sterile soil 1549<br>Water to ²/₃ saturation | 400<br>60 | idem | 10 „ | 2622 | | | 24,09 | 3,37 | 26,08 | | | 96,4 | 13,5 | 104,3 |
| | | | | | | | aver. | 50,52 | 49,48 | 24,30 | 2,81 | 25,18 | 202,1 | 197,9 | 97,2 | 11,3 | 100,7 |
| 2623 | „ | 28 | Sterile soil 1549<br>Water to saturation | 400<br>100 | idem | 10 „ | 2623 | | | 50,41 | 0,10 | 0,00 | | | 121,0 | 0,3 | 0,0 |
| 2624 | „ | 28 | Sterile soil 1549<br>Water to saturation | 400<br>100 | idem | 10 „ | 2624 | | | 52,79 | 0,10 | 0,00 | | | 125,4 | 0,3 | 0,0 |
| | | | | | | | aver. | 48,30 | 51,70 | 51,60 | 0,10 | 0,00 | 116,5 | 123,5 | 123,2 | 0,3 | 0,0 |
| 2627 | „ | 28 | Sterile soil 1783<br>Water to ²/₃ saturation | 400<br>100 | idem | 10 „ | 2627 | | | 16,80 | 0,25 | 23,82 | | | 40,3 | 0,6 | 57,2 |
| 2628 | „ | 28 | Sterile soil 1783<br>Water to ²/₃ saturation | 400<br>100 | idem | 10 „ | 2628 | | | 22,41 | 0,37 | 22,86 | | | 53,8 | 0,9 | 54,8 |
| | | | | | | | aver. | 57,06 | 42,94 | 19,60 | 0,31 | 23,33 | 134,9 | 103,1 | 47,1 | 0,8 | 56,0 |
| 2629 | „ | 28 | Sterile soil 1783<br>Water to saturation | 400<br>144 | idem | 10 „ | 2629 | | | 63,02 | 0,10 | 0,00 | | | 105,0 | 0,2 | 0,0 |
| 2630 | „ | 28 | Sterile soil 1783<br>Water to saturation | 400<br>144 | idem | 10 „ | 2630 | | | 57,42 | 0,12 | 0,00 | | | 95,7 | 0,2 | 0,0 |
| | | | | | | | aver. | 39,67 | 60,33 | 60,22 | 0,11 | 0,00 | 66,2 | 100,5 | 100,3 | 0,2 | 0,0 |
| 2631 | „ | 28 | Sterile soil 1931<br>Water to ¹/₃ saturation | 400<br>60 | idem | 10 „ | 2631 | | | 35,01 | 0,23 | 44,79 | | | 140,0 | 0,9 | 179,2 |
| 2632 | „ | 28 | Sterile soil 1931<br>Water to ¹/₃ saturation | 400<br>60 | idem | 10 „ | 2632 | | | 26,01 | 0,23 | 46,67 | | | 126,4 | 0,9 | 186,7 |
| | | | | | | | aver. | 23,76 | 76,24 | 30,51 | 0,23 | 45,73 | 84,0 | 316,0 | 133,2 | 0,9 | 182,8 |
| 2635 | „ | 28 | Sterile soil 1931<br>Water to saturation | 400<br>180 | idem | 10 „ | 2635 | | | 43,41 | 0,10 | tr. | | | 57,9 | 0,1 | tr. |
| 2636 | „ | 28 | Sterile soil 1931<br>Water to saturation | 400<br>180 | idem | 10 „ | 2636 | | | 45,51 | 0,10 | tr. | | | 60,7 | 0,1 | tr. |
| | | | | | | | aver. | 55,44 | 44,56 | 44,46 | 0,10 | tr. | 73,9 | 59,4 | 59,3 | 0,1 | tr. |

It is seen that there was no trace of nitrification in the saturated soil in the case of two soils and only a trace with the other soil though nitrification proceeded well in all soils when of $^2/_3$ or $^1/_3$ saturation.

In order to have a still more crucial test of nitrifying activity in soil as compared with that in solution, two portions of Omelianski's solution were prepared in the usual way, inoculated; one lot was incubated as a solution, the other portion was absorbed by sterile soil of such quantity as to render the culture about two-thirds saturated. Also an extract of soil with ammonium sulphate added to it in the usual quantity was inoculated and similarly mixed with soil. Suitable control cultures were set up. The results are given in Table 12.

Table 12. Showing amount of nitrification in solution and in the same solution absorbed by soils.

Conditions of experiment 70.

| Sample No. | Date of inoculation | Time of incubation days | Medium | | Inoculum | | Initial nitrogen | |
|---|---|---|---|---|---|---|---|---|
| | | | Kind | Grams | Kind | Grams | Milligrams | Form. |
| 2034 | 2/3/08 | 28 | Omelianski's solut. | 240 | Soil 1867 | 10 | 101,8 | $(NH_4)_2SO_4$ |
| 2035[1] | „ | 28 | Sterile soil 1867 | 400 | Soil 1867 | 5 | 50,9 | „ |
| 2036 | „ | 28 | Extract of soil 1867 | 240 | Soil 1867 | 10 | 101,8 | „ |
| 2038 | „ | 28 | Extract of soil 1867 | 240 | „ | | 101,8 | „ |
| 2037[1] | „ | 28 | Sterile soil 1867 | 400 | Soil 1867 | 5 | 50,9 | „ |
| 2039 | „ | 28 | Sterile soil 1867 | 400 | „ | | 50,9 | „ |

Results of analysis. Experiment 70.

| No. | Percentage of initial nitrogen | | | | | Milligrams of nitrogen in 100 c. c. solut. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unaccounted form | Total recovered | Recovered as | | | Unaccounted form | Total recovered | Recovered as | | |
| | | | Ammonia | Nitrites | Nitrates | | | Ammonia | Nitrites | Nitrates |
| 2034 | 21,60 | 78,40 | 53,50 | tr. | 24,90 | 9,2 | 33,3 | 22,7 | tr. | 10,6 |
| 2035 | 14,01[2] | 114,00 | 0,00 | 0,00 | 114,00 | 6,0[2] | 48,5 | 0,0 | 0,0 | 48,5 |
| 2036 | 37,20 | 62,80 | 47,20 | 1,60 | 14,00 | 16,5 | 26,0 | 20,0 | 0,7 | 6,0 |
| 2038 | 95,70 | 4,30 | 0,00 | 0,40 | 3,90 | 40,8 | 1,7 | 0,0 | 0,2 | 1,7 |
| net | — | — | — | 1,20 | 10,10 | — | — | — | 0,5 | 4,3 |
| 2037 | 11,60[2] | 111,60 | 0,00 | 0,00 | 111,60 | 4,9 | 47,4 | 0,0 | 0,0 | 47,4 |
| 2039 | 58,50 | 41,50 | 19,80 | 0,00 | 21,70 | 24,9 | 17,6 | 8,4 | 0,0 | 9,2 |
| net | — | — | — | 0,00 | 89,90 | — | — | — | 0,0 | 38,2 |

It is seen from the first two numbers that on making proper allowances for the results of the control cultures, nitrification was vastly greater in the solution absorbed by soil than in the same solution not so absorbed, a conclusion which is clearly justified either by basing nitrification upon a percent basis or on the amount of nitrate produced per 100 c.c. of liquid in the medium.

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1) In 2035, 120 c. c. of Omelianski's solution were inoculated with 5 g of soil 1867 and then poured over 400 g of sterile soil. In 2037, 120 c. c. soil extract + ammonium sulphate were inoculated and then poured on soil in similar manner.

2) Excess.

### Nitrifying sewer organisms.

Small quantities of nitrifying earth from the sewer beds of Lawrence, Mass. were obtained through the kindness of Dr. Steven De M. Gage. These samples, thrown into ammonium sulphate solution, nitrified vigorously from the start. A quantity of finely ground coke was placed in a percolator, inoculated with this nitrifying sewer bed material and ammoniacal solution poured through it once or twice daily. In this manner the coke of the percolator soon came to a still more vigorous nitrifying condition.

The effluent from this percolator formed excellent inoculating material for nitrification.

Experiment No. 72. Inoculations were made into soil and into soil extract with the nitrifying effluent from the coke-filled percolator bearing the sewer organisms. The cultures were analyzed at the end of 28 days with the results shown in Table 13.

Table 13. Showing nitrification by sewer organisms in soil and in solution.
Conditions of experiment 72.

| Sample No. | Date of inoculation | Time of incubation days | Medium | | Inoculum | | Initial nitrogen | |
|---|---|---|---|---|---|---|---|---|
| | | | Kind | Grams | Kind | Grams | Milligrams | Form |
| 2051 | 2/8/08 | 28 | Sterile soil 1867 | 390 | Percolator effluent | 10 | 240 | $(NH_4)_2SO_4$ |
| 2052 | ,, | 28 | Sterile soil 1867 | 390 | Percolator effluent | 10 | 240 | ,, |
| aver. | | | | | | | | |
| 2053 | ,, | 28 | Extract of soil 1867 | 300 | Percolator effluent | 10 | 127,3 | ,, |

Results of analysis. Experiment 72.

| No. | Percentage of initial nitrogen | | | | | Milligrams of nitrogen in 100 c. c. solut. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unaccounted form | Total recovered | Recovered as | | | Unaccounted form | Total recovered | Recovered as | | |
| | | | Ammonia | Nitrites | Nitrates | | | Ammonia | Nitrites | Nitrates |
| 2051 | 10,60 | 89,40 | 80,50 | 0,00 | 8,90 | 42,4 | 357,6 | 322,0 | 0,0 | 35,6 |
| 2052 | 19,60 | 80,40 | 73,50 | 0,00 | 6,90 | 78,4 | 321,6 | 294,0 | 0,0 | 27,6 |
| aver. | 15,10 | 84,90 | — | 0,00 | 7,90 | 60,4 | 339,6 | 309,0 | 0,0 | 31,6 |
| 2 053 | 1,80 | 101,80 | 86,50 | 0,00 | 15,30 | 0,8 1) | 43,3 | 36,8 | 0,0 | 6,5 |

These cultures nitrified more vigorously in solutions than any cultures heretofore examined and more vigorously than any of the soils among the many that we have tested during the past two years. Not only this, but basing the nitrification upon the number of grams of nitrogen in the medium, it is seen that there was a larger percent of nitrification in the solution than in the soil culture, a condition which is also without parallel in our experiments with cultures inoculated with soils.

In numerous tests, this sewer culture has proved a vigorous nitrifier in ammoniacal solutions of various compositions; it also nitrifies well in soil.

Here then is, evidently, a nitrifying organism or group of nitrifying organisms, differing radically in physiological relations from those usually found in soils here, in that it can nitrify even better in solutions than in soils.

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1) Excess.

### Test with pure cultures.

Experiment 210. Tests of nitrification in the presence of different amounts of water were also made, using as the inoculum 1 c. c. each of cultures of pure nitrite and nitrate forming organisms[1]) isolated by the Stevens-Temple Silicate medium[2]).

The results are given in table 14.

Table 14. Showing failure of pure culture to nitrify in saturated soils.
Conditions of experiment No. 210.
Nitrogen 240 mgs. os $(NH_4)_2SO_4$.

| Sample No. | Date of inoculation | Days from inoculation to analysis | Medium | | Inoculum | |
|---|---|---|---|---|---|---|
| | | | Kind | Amount | Kind | Amount |
| 2606 | 9/7/08 | 28 | Sterile 1867<br>Water to $^1/_3$ saturation | 400<br>60 | Organism ite<br>ate | 1 c. c. |
| 2607 | „ | 28 | Sterile 1867<br>Water to $^1/_3$ saturation | 400<br>60 | Organism ite<br>ate | 1 „ |
| 2610 | „ | 28 | Sterile 1867<br>Water to saturation | 400<br>180 | Organism ite<br>ate | 1 „ |
| 2611 | „ | 28 | Sterile 1867<br>Water to saturation | 400<br>180 | Organism ite<br>ate | 1 „ |

Results of analysis. Experiment No. 210.

| No. | Percentage of initial nitrogen | | | | | Milligrams nitrogen in 100 c. c. solut. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unaccounted form | Total recovered | Recovered as | | | Initial | Unaccounted form | Recovered | Recovered as | | |
| | | | Ammonia | Nitrites | Nitrates | | | | Ammonia | Nitrites | Nitrates |
| 2606 | | | 45,51 | 0,15 | 12,53 | 400 | | | 182,0 | 0,6 | 50,1 |
| 2607 | | | 46,35 | 0,38 | 11,06 | 400 | | | 185,4 | 1,5 | 44,2 |
| aver. | 42,29 | 57,71 | 45,93 | 0,26 | 11,78 | 400 | 169,1 | 230,9 | 183,7 | 1,0 | 47,2 |
| 2610 | | | 44,11 | 0,07 | 0,00 | 133 | | | 58,7 | tr. | 0,0 |
| 2611 | | | 44,74 | tr. | 0,00 | 133 | | | 59,5 | tr. | 0,0 |
| aver. | 65,53 | 44,47 | 44,47 | 0,04 | 0,00 | 133 | 73,9 | 59,1 | 59,1 | tr. | 0,0 |

Here again there was large nitrification in soil cultures of $^1/_3$ saturation but no nitrification in the saturated soils.

This result is particularly significant in that it shows that the failure to find nitrates in solution and in very wet soils is in no way due to their reduction or destruction otherwise by organisms accidentally present.

### Bases for comparison.

It is difficult to determine what is the most useful basis for comparing bacterial activity in soils with the activity of bacteria in solutions, whether to base the comparison on:

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1) These cultures were pure in the sense that Winogradsky used the term, i. e., they would not render bouillon turbid and they formed nitrite or nitrate respectively from appropriate lower nitrogen compounds.

2) A convenient mode of preparing silicate jelly. (Centralbl. f. Bakt. Abt. II. Bd. XXI. 1908. p. 84.)

1) The percent of nitrogen originally present that is converted into some other form of nitrogen, or:

2) To compare the number of milligrams of nitrogen converted per cubic centimeter of solution with the number of milligrams of nitrogen per cubic centimeter of soil water so converted, or:

3) To compare the number of milligrams of nitrogen converted per cubic centimeter of solution with the number of milligrams of nitrogen converted per gram of moist soil.

To compare the percents of original nitrogen converted in two different media, one weak and the other strong, as in No. 2049 and 2055 of table 4, is manifestly an unfair measure of the nitrifying power of the cultures, since a very weak nitrifier might bring about a nitrification of nearly 100 % in the weak medium, while a very vigorous nitrifier might nitrify to less than 40 % in the more concentrated medium. In such instances, a comparison of the number of milligrams of nitrogen converted per gram of medium or per cubic centimeter of liquid present, is more significant, though this comparison would also be improper, if the nitrification reached such an amount in the weaker medium as to justify the belief that cessation of nitrification was caused by lack of suitable material to work upon.

The nitrifying organisms undoubtedly live and function in the moisture in the soil, so that from the view point of measuring the activity of the organisms, the second method mentioned above seems most useful. This method of comparison, however, probably tends to exaggerate the value of the changes occurring, and from a practical view point, a comparison by the third method is probably the most serviceable, since it pictures approximately the relative nitrifying power of two cultures of equal weight. Each comparison has its special uses and to enable the readers to make such comparisons as are desired, data are given to admit of the use of each of the comparisons suggested.

A comparison of nitrification in soils with nitrification in solutions is probably of very little significance except for the special purpose of this present article, unless some relation can be established showing that the vigor of nitrification in a certain solution can be taken as an index of the nitrifying vigor of the soil used to inoculate the solution.

## General considerations.

The evidence of the tables presented, together with the results of numerous other experiments during our experience of the last two years, forces the conclusion that not all soils which can bring about vigorous nitrification of ammonium sulphate and cottonsed meal in soils in 4 weeks, can nitrify in solutions during that time. Numerous instances show that among the natural soils of this locality few or none can cause nitrification in solutions in 4 weeks, if at all. It is established also that some of these soils which cause nitrification not at all or slightly in Omelianski's, Ashby's, Wiley's, or other ammoniacal solutions, can still nitrify vigorously as soils. It is also brought out that the failure to nitrify is not due to the fact that a small inoculum is used in solution, since in the experiments given in detail there was no nitrification even when 400 grams of the live soil were used as an inoculum and repeated experiments have also shown that the presence of water to or above the point of saturation inhibits nitrification with the soils here used. Notwithstanding this fact, sewer-bed-soil nitrified as well in solutions as it did in soils.

From these facts, it seems that no trustworthy, reliable evidence as to the actual nitrifying power of a soil can be gained by inoculating the soil into solutions in the usual way, unless in the case of soils which are naturally very wet, offering practically the conditions obtaining in a solution. If negative results are had from solution tests, it may well be, as is the case of certain soils of this locality, that a type of nitrifying organisms or composite of organisms is present which is capable of nitrifying in soils but not in solutions; and that though the result is of negative indication, the soil may in reality be a vigorous nitrifier.

If positive results are had, it may be that the soil contains an organism similar to the sewer organisms experimented with, which nitrified more vigorously in solutions than in soils, and that while the indication given by the test is of vigorous nitrifying power, the soil may in reality be of low nitrifying power.

Tests by the method of liquid cultures are not of value even for purposes of comparing the nitrifying power of two soils, since two soils may nitrify to different degrees and yet both give negative results in solutions, or one bearing a vigorous soil-nitrifier may give a negative result and another bearing a vigorous solution-nitrifier, though weak as a soil-nitrifier, may give a strong positive result.

It seems then that to gain a useful indication of the nitrifying vigor of a soil, soil itself must be used as a medium. To picture conditions as they are in the field, the field conditions must be approximated as nearly as possible. With varying soil composition, temperature, light and water relation and a bacterial flora varying in species and vigor, the conditions are very complex and no laboratory tests can ever entirely represent them. It must, however, be the aim of the soil Bacteriologist to approach as nearly as possible the ideal. One imperative step is the testing of the activities of soil bacteria in soils rather than in solutions.

## Summary.

1) Many soils which can nitrify ammonium sulphate and cottonseed meal mixed with them, fail to nitrify ammonium sulphate and cottonseed meal when used as the inoculum for solutions, such as those of Omelianski, Wiley and Ashby.

2) Nitrification is nil or very slight in saturated soils.

3) Nitrification in some soils proceeds as fast as ammonification converting the ammonia to nitrate as fast as it is rendered available by the ammonifying organisms.

4) Nitrification in extract of soil is in some instances very slight as compared with nitrification in the soil itself.

5) Nitrification in soils increases in intensity with the amount of inoculum used.

6) Some nitrifying soils do not nitrify when placed in solutions even though a very large inoculum is used.

7) Nitrifying organisms from sewer beds nitrified better in solutions than in soils.

8) Tests in solutions are not adequate to indicate the nitrifying vigor of a soil.

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